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BIOREMEDIATION TECHNOLOGY FOR TREATMENT OF INDUSTRIAL POLLUTANTS: MICROBIOLOGICAL CONSIDERATIONS

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I. ABSTRACT

This paper reviews the current status of bioremediation technology for the detoxification and degradation of hazardous landfill leachates and groundwaters. Microbial biodegradation of numerous industrial pollutants has been well documented in the literature. Physicochemical factors influencing such biodegradations have been identified, leading to the recent development of several processes for hazardous waste bioremediation. Benefits and limitations of this emerging technology will be reviewed. This technology holds considerable promise for future hazardous waste treatment in Ontario and active research in this area should be aggressively pursued and supported.

II. INTRODUCTION

The Ontario Ministry of the Environment (MOE) has identified 41 priority waste disposal sites in 36 municipalities in Ontario, as having potential to negatively impact the surrounding environment (Sibul, 1987). These, as well as numerous other active and inactive sites, are believed to be releasing hazardous materials (Barker *et al.*, 1987; Proulx *et al.*, 1987) into the environment.

Table 1 summarizes data on the compositional variability of hazardous leachates from several Ontario landfill sites. These leachates include many hazardous chemicals including inorganics (halides and metals), alkanes, aromatics, poly-nuclear aromatics and organochlorines. Many of these compounds have been listed as "priority pollutants" by the U.S. Environmental Protection Agency (EPA; Denit, 1984) and by the MOE (MOE, 1982; MISA,

1987). The variability in leachate composition between the different landfills results from many factors, including the characteristics of the particular surrounding industrial base and the nature of the landfill site (soil type, soil structure, pH, etc.).

In response to the requirement for industry to meet new stringent environmental regulations, there has been a tremendous effort in recent years to develop improved waste treatment processes that are both efficient and cost-effective. The purpose of the study we carried out for the MOE was to review recent developments in the field of biological treatment of hazardous wastes, in order to assess their potential applicability for Ontario landfill leachate remediation (Fein and Yu, 1988). The specific objectives of the study were:

(1) To evaluate the feasibility of both traditional and new biological treatment technologies (relative to conventional physical and chemical processes) for hazardous leachate management;

(2) To identify critical process parameters affecting the suitability of biological treatment of hazardous wastes;

(3) To review recent progress in the development of specialized commercial treatment cultures for industrial pollutant biodegradation, concentrating on natural (i.e. non-genetically engineered) microorganisms;

(4) To provide a risk assessment of biological treatment processes; and

(5) To make recommendations for biological process implementation in Ontario, including timing and identification of research needs.

This paper will focus on the microbiological considerations involved in developing full scale bioremediation processes for waste treatment.

III. OVERVIEW OF BIOREMEDIATION TECHNOLOGY

A variety of aerobic and anaerobic biological waste treatment processes have been employed for years for the treatment of wastes such as sewage and food processing effluents. With the widespread manufacture and use of novel man-made synthetic chemicals (i.e. xenobiotic substances), many of which have been found to be very recalcitrant to conventional microbiological treatments, came the realization that traditional biological processes would have to be refined significantly for the treatment of industrial wastes, landfill leachates and other materials containing hazardous compounds. Significant advances have recently been made in the fields of microbial ecology, metabolism, genetics and bioengineering. This in turn has led to the development and recent introduction of some novel biological treatment processes for xenobiotic wastes.

To date, few full scale installations for the biological treatment of hazardous waste chemicals are operating. Aitken and Irvine (1987) have suggested several reasons for the slow introduction of this technology: (1) the lack of a proven track record as a result of too few full scale operating plants; (2) uncertainty by the regulatory authorities and engineering consultants that these untested systems will consistently meet stringent environmental discharge limits; (3) limitations of laboratory feasibility tests, which are unable to truly duplicate the field environment, to accurately predict full scale field performance. The latter stems largely from our insufficient understanding of the complex microbial ecology involved, and from our incomplete knowledge of the true physicochemical and biological parameters affecting microorganisms in treatment systems.

As a result, Aitken and Irvine point out that many pollution problems that could probably be solved more cheaply and efficiently by biological processes are instead being addressed using the more costly, conventional physical and chemical destruction technologies.

The feasibility of any biological treatment process for handling hazardous environmental pollutants will rely on many non-biological and biological parameters. Effective treatment in any biological treatment process will depend on the ability to provide "suitable" type(s) of microorganisms in the treatment system. The microorganisms must also be maintained in an optimal or satisfactory physiological state to obtain good performance. This will be determined by a host of chemical, physical, nutritional and biological factors which affect microbial growth, survival and metabolic activity (Table 2).

Failure in the past to understand these fundamental ecological principles has impeded the successful development of biological processes for treatment of hazardous wastes. For a particular waste problem, the success of a biological process (i.e. efficiency, kinetics of biodegradation and detoxification, stability, control of undesirable microorganisms, etc.) will depend on how well these factors are understood (and controlled) by the designers and operators of the system.

IV. SURVEY OF BIODEGRADABLE ENVIRONMENTAL POLLUTANTS

A. BIODEGRADABLE ORGANIC POLLUTANTS

Microorganisms have an enormous capacity to degrade natural organic substances, given favourable environmental conditions (Alexander, 1965; Atlas and Bartha, 1987). If microorganisms with the appropriate activities are already present in suitable numbers, biodegradation should begin immediately following the introduction of an organic compound into the environment, or following a short induction period.

The situation with respect to unnatural man-made industrial chemicals, which are being added at a rate of 1,200 chemicals a year to the U.S. EPA's current list of 60,000 industrial chemicals (McCormick, 1985), is more complex. In the environment, such molecules may undergo complete or partial biodegradation or may be entirely recalcitrant to microbiological attack. Also, abiotic forces such as spontaneous chemical breakdown or volatilization can account for partial or complete removal of these compounds (Vogel *et al.*, 1987).

A wealth of laboratory data in the recent scientific literature attests to the fact that many, if not most, xenobiotic chemicals are subject to microbiological attack, given the correct environmental conditions and types of microflora (McCormick, 1985; Johnston and Robinson, 1984). Numerous examples are summarized in Table 3.

The compounds listed in the table include most of the hazardous substances of current environmental concern including industrial chemicals from all of the major groups (halogenated hydrocarbons, polychlorinated biphenyls (PCBs), organic pesticides and herbicides, inorganic pollutants, etc.). From Table 3, it will also be seen that many different types of naturally occurring bacteria are responsible for these diverse activities.

B. RECALCITRANCE OF XENOBIOTIC COMPOUNDS

From a practical consideration, the slow rates of biotransformation and biodegradation for many of the xenobiotic compounds constitute a limitation to the technology. Furthermore, the fact that a xenobiotic compound can be biodegraded in the controlled conditions of the laboratory does not necessarily mean that the same process will occur in a field situation. Conditions are likely to be drastically different in terms of physicochemical, hydrological and biological parameters, compositional complexity and strength of the particular waste, as well as scale of operation (McCormick, 1985; Aitken and Irvine, 1987). If the field conditions are not correct, xenobiotic chemicals may be quite resistant to microbial attack and persist for many years.

The term mineralization is used to describe complete microbiological biodegradation of organic materials to carbon dioxide, water and simple inorganic substances. For many xenobiotics, however, biodegradation is only partial, leading to the production of recalcitrant intermediates which may have lower, higher or similar toxicity to the original chemical (Bartha, 1969; Summers and Silver, 1978; Sundstrom, 1982; Roberts, 1987).

Other factors, in addition to unfavourable environmental conditions, are responsible for biological recalcitrance of many xenobiotic compounds, including:

(1) Unnatural chemical substitutions (e.g. chlorination, highly condensed aromatic rings, unusual bonds, etc.) which make the molecules unreactive (e.g. due to steric hindrance, strong electronegativities of halogen substituents) and/or unrecognizable by microbial enzymes (Steiert and Crawford, 1985);

(2) Failure of the xenobiotic substance to induce the synthesis of catabolic enzyme(s) which would degrade it;

(3) Inability of the xenobiotic substance to enter the microbial cell, due to lack of specific transport proteins (permeases);

(4) Limited bioavailability of the xenobiotic substance to the cell;

Bioavailability can be reduced as a result of immobilization of molecules onto inorganic surfaces (e.g. clay particles) or organic particles through adsorption or ionic interactions. Similarly, with highly lipophilic pollutants such as PCBs and other chlorinated hydrocarbons, solubilization and partitioning of the molecules into biological membranes (e.g. in biological sludge) can restrict their accessibility to microbial catabolic processes (Wierich and Gerike, 1981; Richards and Shieh, 1986; Paris *et al.*, 1977; Johnston and Robinson, 1984).

(5) Severe toxicity of the pollutant or its metabolic intermediates for microorganisms.

The cell membranes of microorganisms can concentrate lipophilic toxicants, leading to a 10 to 1000 fold increase in the concentration of these substances in the cells. In biological wastewater treatments, accumulation of hazardous, poorly metabolized lipophilic pollutants into microbial sludges complicates the downstream handling of the sludge. These sludges must be considered as hazardous and should be disposed of accordingly. When evaluating biotreatability data, the investigator must consider the possibility of bioaccumulation, since this phenomenon can remove lipophilic pollutants from the aqueous assay system. This could lead to an overestimation of the extent and rate of biodegradation unless proper chemical mass balances are conducted (Aitken and Irvine, 1987).

C. SIGNIFICANCE OF MICROBIAL COMMUNITIES IN BIODEGRADATION

Numerous laboratory studies have shown that pure microbial cultures (i.e. single species) can attack and degrade many xenobiotic substances. It is important to realize however that, in both natural ecosystems and full scale waste treatment systems, biodegradation is usually mediated by microbial communities containing different types of microorganisms having many different metabolic capabilities. Pure microbial cultures are rarely encountered in natural environments (Slater and Lovatt, 1984).

Many naturally occurring microbial communities capable of biodegrading various xenobiotic substances have been isolated and characterized (Table 4). In an excellent review on the significance of microbial communities on biodegradation, Slater and Lovatt (1984) point out that individual members of these communities are often much less effective than the community as a whole in their abilities to biodegrade many xenobiotic pollutants. This microbial synergy results in significantly enhanced rates of biodegradation and mineralization or biotransformation of a much wider range of xenobiotics than is possible by any of the single species in a community. The term "consortium" has been introduced to describe a two-membered culture or natural assemblage such as those in Table 4 in which each microorganism derives benefit from the other as a result of their interactions (Brock *et al.*, 1984).

For biological waste treatment processes to operate successfully in the field, stability of the microbial communities is a prime need. Any of a number of critical physicochemical or biological factors (see Table 2) can upset the stability of the community and hence, the efficacy of biodegradation. If these parameters are not properly understood and controlled, costly process failures and shutdowns may result.

A wide variety of microbial interactions can affect the performance of a waste treatment process, including commensalism, mutualism, competition and predation (Atlas and Bartha, 1987). Commensalistic or mutualistic interactions may promote biodegradation processes by a number of mechanisms. Nutritional assistance is one means whereby one member of a microbial community provides another with a required growth nutrient. For example, Jenson (1957), characterized a commensalistic microbial community, enriched from soil, which biodegraded trichloroacetic acid (TCA). The community consisted of three microorganisms, an unidentified bacterium (strain 3C1) which could dechlorinate TCA and use it as a primary carbon and energy source for growth, and two species of *Streptomyces*, which were not able to grow on TCA. Strain 3C1 could not grow in the absence of the *Streptomyces* spp., unless vitamin B_{12} was added. In the natural commensalistic culture growing on TCA, it was concluded that one or both of the *Streptomyces* spp. provided strain 3C1 with the essential vitamin while using substrates other than TCA as their carbon and energy sources.

Other mechanisms for nutritional assistance in mutualistic or commensalistic communities include: (1) increasing the availability of a bound or otherwise inaccessible substrate, for example, by excreting a biosurfactant to release an insoluble pollutant (Banerjee *et al.*, 1983; McCormick, 1985); (2) release of extracellular enzymes whose activities provide readily utilizable substrates from otherwise non-available complex substrates (e.g. glucose from cellulose); and (3) production of utilizable substrates through co-metabolism (discussed below).

The detoxification (through biodegradation or biotransformation) of toxic pollutants in an environment is another important mechanism whereby one member of a mutualistic or commensalistic community can assist another and promote the overall efficacy of biodegradation in a biological treatment process.

D. CO-METABOLISM

Co-metabolism, or fortuitous metabolism as it is sometimes called, is a phenomenon whereby a bacterium is able to partially or totally transform a non-growth substrate (i.e. a specific xenobiotic compound) in the obligate presence of another organic compound which serves as its growth substrate (i.e. carbon and energy source). It is a fortuitous form of metabolism in that the reactions are catalyzed by microbial enzymes which have broad substrate specificity (Johnston and Robinson, 1984).

In the case of partial transformation, the chemical intermediates produced by co-metabolism may be available for consumption as carbon and energy sources by other members of the microbial community. For example, the bacterium *Mycobacterium vaccae* is able to co-metabolize cyclohexane while growing on propane, but cannot assimilate either the cyclohexane its co-metabolic by-product, cyclohexanone (Beam and Perry, 1974). The latter, however, can be used by other bacteria in this commensalistic community, which also cannot utilize the cyclohexane.

Although many laboratory examples of co-metabolism have been reported in the literature, the true extent to which it occurs in nature is not clear (Johnston and Robinson, 1984). It's exploitation has been considered as a possible approach to the *in situ* treatment of pollutants, whereby acclimatized microbial seed cultures would be injected along with a growth substrate into a suitable aquifer system (Jhaveri *et al.*, 1983; Wilson and Ward, 1987). It would appear that this approach is being successfully applied to a number of hazardous waste problems, including a few of the so-called Superfund sites in the United States (A. Bourquin, personal commun.). However, as pointed out by McCarty (cited by Roberts, 1987), the economic feasibility of injecting huge quantities of an energy source (i.e. primary substrate) into the ground must be carefully evaluated for each waste treatment situation. Primary energy sources are required at levels of about 100 to 1000 times greater than that of the pollutants if the latter are to be co-metabolized. Thus, for every kilogram of hazardous chemical to be transformed, 100 to 1000 kg of primary substrate would have to be added to the aquifer. The operating costs for such treatment could be enormous! At a Superfund site in Florida being treated *in situ* by ECOVA Corp., the high content of natural organic compounds in the contaminated soil appears to be providing much of the required organic nutrient for the co-metabolizing bacteria (A. Bourquin, personal commun.)

E. GENETICALLY IMPROVED STRAINS

Microbiologists have shown that, for many recalcitrant pollutants, it is possible to develop genetically improved or acclimatized seed cultures (single or mixed species) having an increased capacity to biodegrade or detoxify these compounds. The rationale behind this work is that, by developing a number of specific superior strains and then mixing and matching them, it may be possible to customize microbial preparations for combating specific hazardous waste problems (Gasner, 1979; McCormick, 1985). In particular penta- and tetra-chlorodibenzodioxins (TCDDs) and their dibenzofuran analogues (TCDFs), PCBs, toxaphenes, Dieldrin/Aldrin, Heptachlor and its epoxide, the Chlordanes, Mirex, Kepone and the hexachlorocyclohexane insecticides, have been identified, as deserving high priority attention for research into improving their biodegradability (Johnston and Robinson, 1984). All are highly toxic and persistent in the environment.

Laboratory methods that have been successfully applied include conventional strain isolation and enrichment (acclimatization and adaptation), classical mutagenesis, plasmid-assisted breeding and genetic engineering (Aitken and Irvine, 1987; Chakrabarty, 1974; Johnson *et al.*, 1985; Johnston and Robinson, 1984; Kellogg *et al.*, 1981; Kilbane *et al.*, 1982; McCormick, 1985; Roberts, 1987; Schlegel and Jannasch, 1967; Steiert and Crawford, 1985; Veldkamp, 1974;). Further discussion of these methods and their application to the development of better degradative microorganisms for bioremediation processes are provided in our full report to MOE (Fein and Yu, 1988).

When developing a suitable seed culture for bioaugmentation, regardless of the approach to be taken, there are a number of physiological properties which need to be considered as targets for possible improvement. Table 5 presents a summary of those traits which are desirable or essential for a worthy commercial seed preparation.

A number of suppliers presently are selling natural or acclimatized bacterial seed preparations which they claim offer superior performance for treating certain types of wastes including greases, phenols or even "dioxin herbicide" (Johnson *et al.*, 1985, Gasner, 1979). In many cases, however, the claims made for these microbial cultures appear to be highly exaggerated, unsubstantiated and scientifically questionable (Johnston and Robinson, 1984).

On the other hand, significant progress has been made over the past fifteen years in the development of microorganisms which exhibit enhanced biodegradative abilities towards specific pollutants. The challenge now is to successfully move the technology out of the laboratory and into the field. As seen in Table 2, to be effective in the field a seed microorganism or mixture of seed microorganisms must display a range of physiological properties. The capacity to degrade a particular pollutant in a bioreactor or field situation will be of limited benefit if the microorganisms are susceptible to the toxic effects of other pollutants that might also be present in the waste. With most hazardous wastes, such as industrial landfill leachates, complexity and high toxicity are often the norm (Johnston and Robinson, 1984; Barker *et al.*, 1987; Roberts, 1987).

Furthermore, in all real world treatment situations, the seed microorganisms must successfully compete with the natural indigenous

microflora for available resources (nutrients, attachment sites, space, etc.) if they are to establish themselves and effectively catalyze the desired transformations. This in turn will depend on how well the bacteria in the seed preparation can cope with the particular set of conditions imposed by each waste treatment problem. We are still a long way from achieving this objective. Where commercial seed cultures have been effective, continued performance almost always requires that these preparations be readministered to the treatment system on a repeated basis, to maintain the desired level of activity (Johnson *et al.*, 1985). This constrasts sharply with the more economically desirable "one shot" starter culture approach.

For *in situ* treatments of hazardous waste landfills, this problem is magnified since very few of these parameters can be controlled and because of variation in site geology (soil structure and composition, hydrology, etc.). The most important constraint on biodegradation in soils is thought to be the inaccessibility of the pollutants to the microorganisms, as a result of adsorption and other abiotic factors that were discussed earlier. Furthermore, the conditions in such environments are often extreme for microorganisms, being highly toxic due to high concentrations of toxic metals and halogenated compounds and having an unusually low water activity (Johnston and Robinson, 1984). What works well on one particular waste may not work so well on a similar waste at a different treatment site. With above-the-ground treatment, the problem may be somewhat less severe, as operators can control and optimize many of the critical physicochemical factors. Variability in the xenobiotic composition of the wastes, however, will always be a significant factor limiting universal applications of any specifically developed microbial preparation.

The solution to such complex problems may lie in the development of large libraries of biodegradative microorganisms from which specific mixed cultures could be formulated to suit a particular waste problem (McCormick, 1985). Until more is learned about the ecological aspects of biological waste treatment, this goal will not be realized. Furthermore, such libraries will probably have to be quite large, since bacteria capable of degrading one member of a class of xenobiotic substances (e.g. PCBs, toxaphenes or halobenzoates) are often unable to biodegrade another. For example, with benzoic, *p*-hydroxybenzoic and salicylic acids, which differ only in a single hydroxyl substitution, biodegradation of each is mediated by different strains using distinct catabolic pathways (Johnston and Robinson, 1984).

V. FUTURE OUTLOOK

We have seen that microorganisms have an enormous capacity to biodegrade or transform numerous xenobiotic substances, given the right environmental conditions. As was discussed, many interacting factors will ultimately determine the environmental fate of these compounds. At the applied level, the research and development challenge is how to best exploit microorganisms and transfer the emerging technology from the laboratory environment to full scale operational field processes.

Over the past several years we have seen the introduction of a number of pilot scale and full scale biological treatment systems for remediation of contaminated industrial effluents, landfill leachates and groundwaters. Examples include conventionally designed aerobic (e.g. aeration lagoon, activated sludge, etc.) and anaerobic (e.g. anaerobic sludge blanket reactor, anaerobic filter, etc.) systems, as well as a number of newer process designs (e.g. *in situ*, solid, and slurry-phase soil treatment processes, sequencing batch reactor, the hybrid PACT system, etc.). Some of the latter systems are being currently tested at several heavily contaminated landfills in the United States (i.e. Superfund sites) with apparent success. For further discussion of these various processes including references, the reader is referred to our full report to MOE (Fein and Yu, 1988).

Bioremediation holds considerable promise for hazardous waste treatment, and offers many potential advantages over comparable non-biological treatment technologies in current usage. These biological treatments are often quicker and more cost effective than comparable physicochemical processes in current usage. Bioremediation warrants serious consideration for Ontario industrial pollution problems, both as a stand alone technology and as a unit operation to be used in conjunction with other current technologies. The choice of the most suitable process design for a particular pollution problem can only be determined after thorough feasibility analysis both and in the laboratory and on-site.

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TABLE 1

CONTAMINANTS FOUND IN LANDFILL LEACHATES[a]

Contaminant	Landfill Sites[b]					
	Borden	Woolwich	North Bay	New Borden	Upper Ottawa Street	Tricil
Aliphatic, aromatic and carboxylic acids	20	>10,000	>300	--	>1000	--
Carbon tetrachloride	<1	5	<1	9	p	n.d.
Chloroform	<1	20	<1	25	5	n.d.
Trichloroethylene	1	37	2	750	p	n.d.
Trichloroethane	n.d.	7	<1	90	20	8.44
Tetrachloroethylene	n.d.	2	<1	<1	<1	n.d.
Acetone	<1	-	6	-	6	-
Tetrahydrofuran	p	-	9	-	200	-
1,4-Dioxane	n.d.	-	<1	-	p	-

(continued)

TABLE 1 (Cont'd)

CONTAMINANTS FOUND IN LANDFILL LEACHATES

Contaminant	Landfill Sites					
	Borden	Woolwich	North Bay	New Borden	Upper Ottawa Street	Tricil
Benzene	3	70	51	50	60	7920
Toluene	1	7500	60	1400	2600	9520
Xylenes	<1	700	140	500	3500	-
Ethylbenzene	<1	1100	64	120	700	3320
Tetramethylbenzene	n.d.	10	250	70	450	-
Chlorobenzene	n.d.	n.d.	105	n.d.	110	-
Dichlorobenzenes	<1	n.d.	13	n.d.	5	-
Naphthalene	n.d.	50	15	260	p	2350
Phenols	-	1100	10	p	p	-
Benzothiozoles	<1	30	10	n.d.	p	-
PAH's	-	-	n.d.	-	n.d.	-
Phthalates	<1	p	110	p	p	-

[a]Source: Barker *et al.*, 1987.
[b]The approximate concentrations (mg/L) of selected organic contaminants found in leachate - contaminated groudwaters at six landfill sites.
p = detected but concentration not estimated
n.d. = not detected
- = not determined

TABLE 2

PHYSICAL, CHEMICAL AND BIOLOGICAL FACTORS

INFLUENCING MICROBIAL BIODEGRADATION

I. Physical Parameters

- a) Temperature
- b) Alkalinity/pH
- c) Moisture (Water Activity)
- d) Salinity
- e) Oxygen Availability
- f) Redox (Eh)
- g) Soil Structure and Hydrology
  - i. Pollutant Bioavailability
    -adsorption/ionic binding
    -solid/liquid interfaces
    -pollutant spatial distribution in ecosystem

II. Chemical Parameters

- a) Organic Composition and Strength of the Waste Feedstocks, incl.
  - i. Pollutant Composition
  - ii. Pollutant Complexity
  - iii. Pollutant Concentration
- b) Presence of Toxicants
  - i. Toxic Pollutants
  - ii. Toxic Metabolic Intermediates
  - iii. Heavy Metals
- c) Nutrient Availability
  - i. Nitrogen
    -carbon:nitrogen balance
  - ii. Macronutrients (magnesium, potassium, phosphorous and sulfur)
  - iii. Micronutrients
    -trace elements (e.g. iron, zinc and nickel)
    -essential growth factors (e.g. vitamins, amino acids, etc.)

TABLE 2 (Cont'd)

PHYSICAL, CHEMICAL AND BIOLOGICAL FACTORS

INFLUENCING MICROBIAL BIODEGRADATION

III. Biological Factors

- a) Microbial Interactions
  - i. Mutualism, Competition, Predation etc.
- b) Seed Cultures (Bioaugmentation)
  - i. Dose (Inoculum) Size and Frequency
- c) Bioaccumulation Affecting Bioavailability

TABLE 3

EXAMPLES OF POLLUTANT-DEGRADING MICROORGANISMS AND SUBSTRATES[a]

MICROORGANISM	POLLUTANT(S)
Achromobacter	halogenated hydrocarbons, hydrocarbons, phenoxyacetates, *tert*-butylbenzene
Acinetobacter, *Actinomucor elegans*	diethyleneglycol, hydrocarbons, PCB
Actinomycetes	petroleum
Aerobacter aerogenes	petroleum hydrocarbons
Aeromonas	benzene
Agrobacterium	phenanthrene
Alcaligenes	halogenated hydrocarbons
	halogenated hydrocarbons, hydrocarbons, linear alkylbenzene sulfonates, PCB, polycyclic aromatics
Arthrobacter	benzene, Diazinon, hydrocarbons, pentachlorophenol, phenoxyacetates, polycyclic aromatics, pulp mill lignins, various phenols
Aspergillus	carbamate, malathion, phenoxyacetates, pulp mill lignins, various phenols
Azotobacter	catechol
Bacillus sphaericus	aromatics, long-chain alkanes
Beijerinckii	phenylureas
Brevibacterium	anthracene, biphenyl
	2,4,5,-T, aromatics, halogenated hydrocarbons, phenanthrene, polycyclic aromatics
Candia tropicallis	PCB
Chaetomium globosum	alachlor
Chlorobium thiosulfatophilum	waste gypsum
Chromobacter	pulp mill lignins, various phenols
Cladosporium	petroleum hydrocarbons
Clostridium	halogenated hydrocarbons

(continued)

TABLE 3 (Cont'd)

EXAMPLES OF POLLUTANT-DEGRADING MICROORGANISMS AND SUBSTRATES[a]

MICROORGANISM	POLLUTANT(S)
Corynebacterium	halogenated hydrocarbons, phenoxyacetates
Cunninghamella elegans	PCB
Desulfobacter postgatei	waste gypsum
Escherichia coli	organophosphates
Flavobacterium	hydrocarbons, pentachlorophenol
Fusarium solani	propanil
Geotrichum marinum thirumalachar	petroleum
Helminthosporium	carbofuran
Hydrogenomonas	halogenated hydrocarbons
Hyphomicrobium	sodium methyl sulfate, etc.
Klebsiella	PCB, toluene
Micrococcus	branched hydrocarbons, hydrocarbons
Moraxella	benzene
Mycobacteria	aromatics, branched hydrocarbons, cycloparaffins
rhodochrous	benzene
Nocardia	hydrocarbons, monoalkylbenzenes, naphthalene, phenoxyacetates, polycyclic aromatics
Propionibacterium pentocaceum	organophosphates
Pseudomonas	4-alkyltoluenes, alkyammonium, alkylamine oxides, anthracene, benzene, heavy metals, hydrocarbons, malathion, methylnaphthalenes, naphthalene, organophosphates, p-cumene, p-xylene, parathion, PCB, pentachlorophenol, phenanthrene, phenoxyacetates, phenylureas, polycyclic aromatics, rubber, secondary alkylbenzenes;

(continued)

TABLE 3 (Cont'd)

EXAMPLES OF POLLUTANT-DEGRADING MICROORGANISMS AND SUBSTRATES[a]

MICROORGANISM	POLLUTANT(S)
aeruginosa	oleaginous materials, pulp by-products
cepacia	halogenated hydrocarbons, 2,4,5-T
cepacia var. niagarous	halogenated hydrocarbons
fluorescens	chromates, surfactants
paucimobilis	cyanides, thiocyanates
putida	phenolics, toluene
strutzeri	orcinol
Rhizoctonia solani	alachlor, phenylurea
Rhizopus	PCB
Scolecobasidium	petroleum hydrocarbons
Serratia marascens	bis (2-ethylhexyl) phthalate
Strain DCBI (Tiedge)	chlorobenzoate
Streptococcus faecalis	organophosphates
Streptomyces	diazinon, halogenated hydrocarbons, phenoxyacetates
Thiobacillus ferroxidans	sulfur
Trichosporon	pulp mill lignins, various phenols
Xanthomonas	hydrocarbons, polycyclic aromatics, pulp mill lignins, various phenols
Zylerion xylestrix	cyclodiene type pesticides/ herbicides

[a]Sources: McCormick, 1985; Ouellette and Cheremisinoff, 1985.

TABLE 4

BIODEGRADATION ENHANCED BY THE INTERACTION OF MICROBES[a]

ORGANISMS	COMMENTS
Hydrogenomonas sp. and *Arthrobacter* sp.	A. grew on p-chlorophenylacetic acid produced from DDT[b] by H.
Pseudomonas sp. and *Achromobacter* sp.	The pair grew on Silvex[c], but not the individual strains. Chloride, CO_2 and a small amount of 2,4,5-trichlorophenol was produced.
Bacillus polymyxa and *Proteus vulgaris*	P. produced nicotinic acid required by B.; B. produced biotin required by P.
Chlorobium limicola and *Desulfovibrio* sp.	C. used HS^- as electron donor producing SO_4^{2-}; D. used SO_4^{2-} as electron acceptor, producing HS^-.
Saccharomyces cerevisiae and *Lactobacillus casei*	S. produced riboflavin required by L.
Nitrosomonas sp., *Nocardia altantica* and *Pseudomonas* sp.	The conversion of NH_4^+ to NO_2^- by Nit. increased in the presence of Noc. and P.
Lactobacillus plantarum and *Streptococcus faecalis*	L. produced folic acid required by S.; S. produced phenylalanine required by L.
Pseudomonas sp., unidentified bacterium, *Trichoderma viride*, *Ps. putida*, budding yeast, *Flavobacterium* sp. and unidentified *pseudomonad*	The first four organisms were primary degraders of Dalapon[d]. The others grew on the waste products of the first four.

TABLE 4 (Cont'd)

BIODEGRADATION ENHANCED BY THE INTERACTION OF MICROBES[a]

ORGANISMS	COMMENTS
Methanobacterium sp. and "S" organism	"S" fermented ethanol to H_2; M. used H_2 for growth and methane formation.
Methanobacterium sp. and *Desulfovibrio desulfuricans*	D. released acetate which was fermented by M. to produce methane.
Pseudomonas putida and *Pseudomonas* sp.	The mixed culture used polyvinyl alcohol as sole carbon source, the isolated strains could not. Ps. sp. degraded PVA but required growth factors from P. put.; P. put. grew on PVA metabolites from Ps. sp.
Three pseudomonads and *Hyphomicrobium* sp.	The pseudomonads oxidized methane; H. used the methanol, preventing its accumulation to levels that inhibit the pseudomonads.

[a]Data complied by Johnston and Robinson, 1984
[b]DDT = 1,1,1-Trichloro-2,2-bis[*p*-chlorophenyl]ethane
[c]Silvex = 2-[2,4,5-Trichlorophenoxy]propionic acid
[d]Dalapon = 2,2-Dichloropropionic acid

TABLE 5

CHARACTERISTICS OF AN IDEAL COMMERCIAL SEED CULTURE

1. Increased expression/activity of degradative pathways.
2. Increased substrate utilization range.
3. Ability of seeded microorganisms to successfully compete/establish themselves in the intended ecosystem.
4. Tolerance of toxicants in the intended ecosystem.
5. Ease of handling and application.
6. Shelf life stability.
7. Non-pathogenic/free of risks.
8. Cost-effective (low dose size and low application frequency).
9. Readily available from supplier.